COMPARATIVE CHROMATOGRAPHIC ANALYSIS AND PHARMACODYNAMIC ACTIVITIES OF AQUEOUS MUSHROOM EXTRACTS (PLEUROTUS TUBER-REGIUM) WITH OTHER ANTIGLAUCOMA DRUGS.

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ABSTRACT

Experimental studies have shown that aqueous extract of *Pleurotus tuber-regium* (PT) caused a reduction in steroid induced ocular hypertension in feline models and a contractile effect on isolated bovine iris. A comparative thin layer chromatographic study and Ultraviolet-Visible spectrophotometric analysis (TLC-UVS) of PT in comparison with known antiglaucoma drugs (0.5% betaxolol, 0.5% timolol maleate, 2% pilocarpine, and 0.005% latanoprost) was carried out to identify their similarities. Graded concentrations (5mg/ml, 10mg/ml, 20mg/ml and 40mg/ml) of PT were spotted along with the drugs on prepared glass plates. Silica type 60 gel served as the stationary phase while a solvent system of 50ml chloroform and 50ml absolute ethanol was used as the solvent system. On completion, comparison was made between the extracts and the drugs based on their migration speeds and retardation factors. Further analytic studies were carried out on the filtrates gotten from thin layer chromatography with an Ultraviolet-Visible spectrophotometer. Thin layer chromatographic (TLC) analysis showed that the extracts compared favorably with the drugs under study, particularly with timolol maleate and latanoprost, as they had similar migration distances and retardation factors (R_f). Spectrophotometry revealed that the extract has an absorption spectrum within the ultra-violet wavelength range with a λmax of 260nm, this is comparable to the known absorption spectra of the anti-glaucoma drugs. PT may likely have similar substances with known anti glaucoma drugs. This is a preliminary evaluation of the crude aqueous extract of PT.

Key Words: *Antiglaucoma drugs, Chromatographic Analysis, Pharmacodynamics, Pleurotus tuber-regium.*

INTRODUCTION

Mushrooms are manna and their water heal eye diseases[1], *Pleurotus tuber-regium* (PT), a gilled fungus found in the tropics of Africa, Asia and Australasia is cultivated for food and for treatment of a wide range of disorders (e.g., headache, stomach pain, fever and colds). There are some evidences of its effects on high blood pressure, diabetes, tumors, fungal and bacterial infections ([2-9]). Phytochemical analysis shows that it contains alkaloids, saponins, flavonoids, tannins, anthraquinnone and phytates[10]. Topically administered aqueous extract of PT reduces induced ocular hypertension in cats. Experimental studies have shown that aqueous extract of *Pleurotus tuber-regium* (PT) caused a reduction in steroid induced ocular hypertension in feline models and a contractile effect on isolated bovine iris. ([11-13]). It was reported that Garcinia kola extracts showed corresponding sites of separation with known antiglaucoma drugs on TLC.

Glaucoma is the second leading cause of blindness globally. The highest prevalence of open angle glaucoma occurs in blacks. There are an estimated 60 million people with glaucomatous optic neuropathy and an estimated 8.4 million people who are blind as a result of glaucoma[15]

Thin layer chromatography is a method of separating non-volatile mixtures. It is used to identify compounds present in a given sample. The retardation factor (R_f), defined as the distance travelled by the compound divided by the distance travelled by the solvent can provide corroborative evidence as to the identity of a compound[16]. If the identity of a compound is suspected but not yet proven, an authentic sample of the

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compound or standard is spotted side by side with the compound in question[17]. If two substances have the same (R_f) value, they are likely (but not necessarily) the same compound. This forms the basis of the comparative analyses of the antiglaucoma drugs with the extracts. In this study thin layer chromatographic and ultra-violet spectrophotometric analysis (TLC-UVS) of PT in comparison with known antiglaucoma drugs (0.5% betaxolol, 0.5% timolol maleate, 2% pilocarpine, and 0.005% latanoprost) was carried out to show if they are identical.

METHODS

The graded concentrations of PT to be analyzed were 5mg/ml, 10mg/ml, 20mg/ml and 40mg/ml. The anti-glaucoma drugs under comparison were 0.5% Betaxolol hydrochloride, 0.5% Timolol maleate, 2% Pilocarpine hydrochloride and 0.005% Latanoprost. The selected anti-glaucoma drugs to be used in the study were basified with ammonium hydroxide to render them preservative free (**Adapted from Adefule *et al.*, 2008).**

Type 60 silica gel known as amorphous silica gel 60 served as the stationary phase. A solvent system of 50ml chloroform and 50ml absolute ethanol served as the mobile phase. The prepared plates were spotted with the graded concentration of PT side by side with the antiglaucoma drugs under comparison and left for 1 hour for the separation.

The plates were then removed from the TLC chamber and left to dry completely. After this, they were put in the iodine tank for another 45 minutes. Photographs of these plates were taken immediately after being removed from the iodine tank; studied and analyzed for identification sites of migrations across the plates. The retardation factor was then calculated for the spots and comparisons made.

Spectrophotometry Analysis

The Ultraviolet-Visible spectrophotometer was used to determine the absorption spectrum of the analyte, *PT* extract, and the anti-glaucoma drugs that was scraped off from the TLC plates (qualitative analysis). The absorption spectrum is a plot of absorbance versus wavelength. Compounds absorb light strongly at a particular wavelength range. This is characteristic for each compound. Hence, this serves as a method of identifying and comparing the active ingredients found in aqueous extracts of PT with those found in the antiglaucoma drugs. The plot reveals the wavelength at which absorbance is maximum ((λmax), which is also an identifying mark for each compound.

RESULTS

A sample of the chromatographic plates is shown in Plate 1 with its migration distance and retardation factor (Table 1). The average retardation factor (Table 3) for the five plates was calculated from the different migration distances (Table 2) as shown below:

Retardation factor and Migration distances

Retardation factor equals the migration of the distance of the substance divided by the migration of the solvent front. Mathematically:

Rf=Ds/Df

Where:

Rf = retardation factor

Ds = migration distance of substance

Df = migration distance of solvent front

NB: the distance of the solvent front (Df) of both plates = 15cm

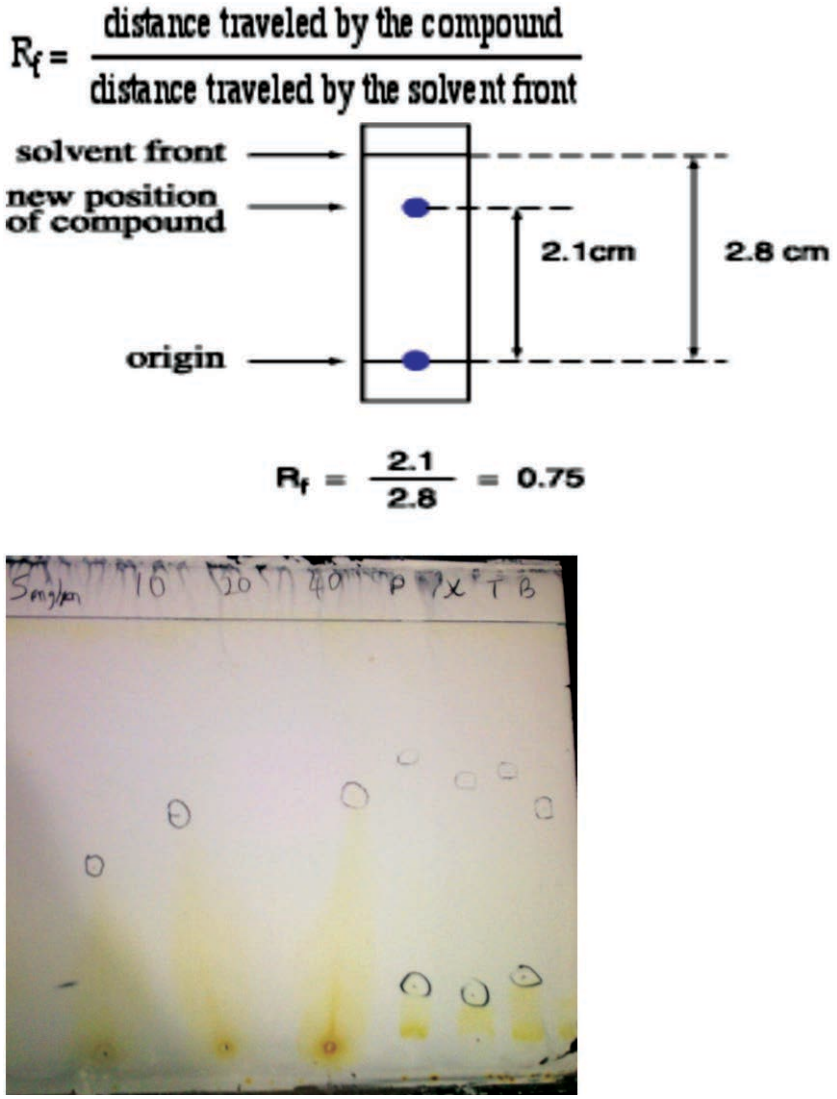


Plate 3: Chromatographic analysis of preservative free 2% Pilocarpine (P), 0.5% Timolol (T), 0.5% Betaxolol (B) and 0.005% Latanoprost (X) with graded concentrations of Pleurotus tuberregium (5mglml, 10mg/ml, 20mg/ml and 40mg/ml). At the bottom of the plates are the spots of the samples, while the extracts concentration and drug under comparison are written at the top of the plates.

Table 1: Migration distances of various concentrations of PT and 2% Pilocarpine, 0.005% Latanoprost, 0.5% Timolol, and 0.5% Betaxolol for Plate.

Samples	Migration distance	Retardation Factor
5mg/ml	5.50cm	0.366
10mg/ml	6.50cm	0.433
20mg/ml	8.00cm	0.533
40mg/ml	9.50cm	0.633
2% Pilocarpine	9.40cm	0.626
0.05% Latanoprost	10.40cm	0.693
0.5% Timolol	9.30cm	0.620
0.5% Betaxolol	4.00cm	0.266

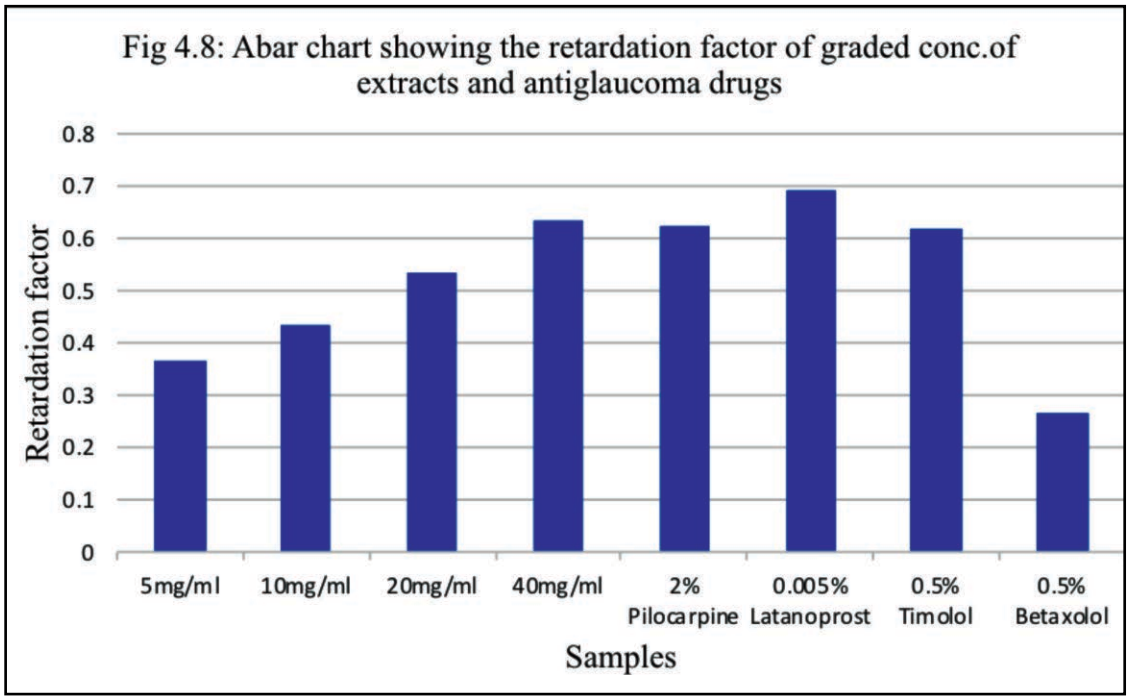


Fig 1: A bar chart showing the retardation factor of graded concentration.of extracts and antiglaucoma drugs.

Table 2: Summary of plate 1—5, showing the mean values of the migration speeds of the various concentrations of PT extract and Antiglaucoma drugs.

Sample	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Mean
5mg/ml	5.80cm	5.70cm	5.50cm	5.60cm	-------	5.65cm
10mg/ml	6.20cm	6.80cm	6.50cm	6.60cm	6.80cm	6.58cm
20mg/ml	7.00cm	7.50cm	8.00cm	7.90cm	7.80cm	7.64cm
40mg/ml	10.50cm	9.80cm	9.50cm	9.40cm	10.00cm	9.84cm
2% Pilocarpine	9.20cm	9.20cm	9.40cm	9.40cm	----------	9.30cm
0.5% Betaxolol	---------	---------	4.00cm	4.50cm	-----	4.25cm
0.005% Latanoprost	----------	----------	10.40cm	10.20cm	10.30cm	10.3cm
0.5% Timolol	9.25cm	----------	9.30cm	9.20cm	9.10cm	9.21cm

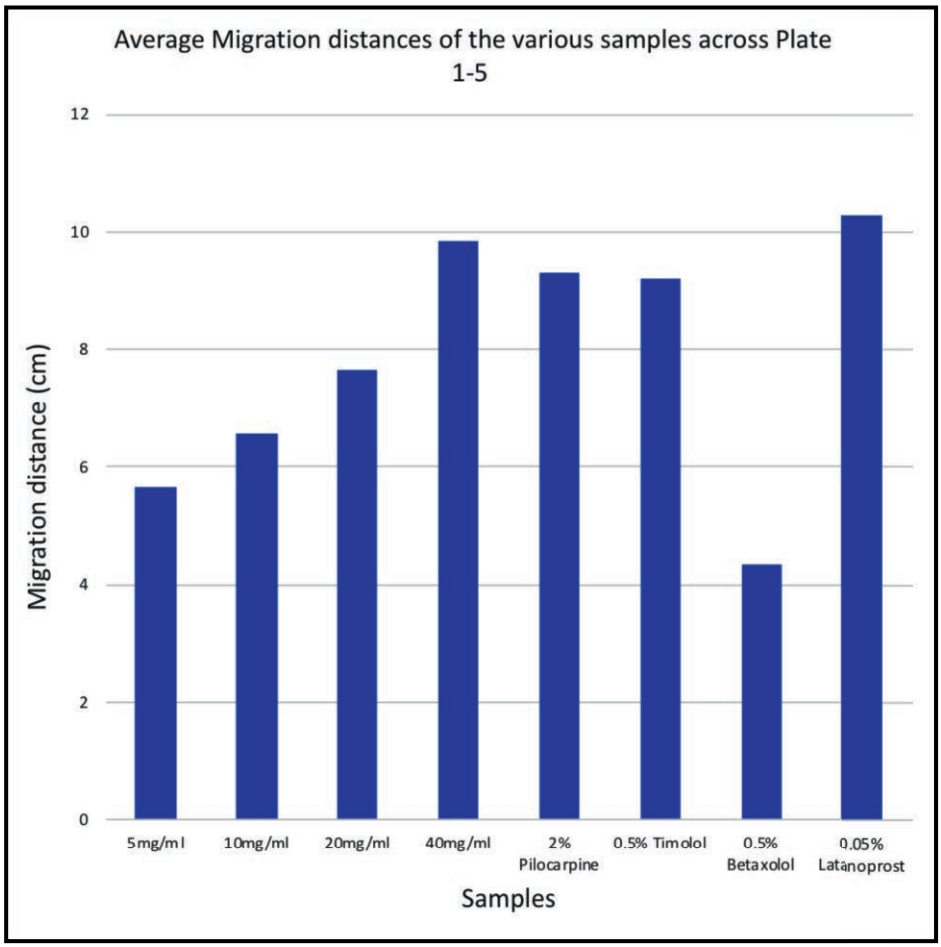
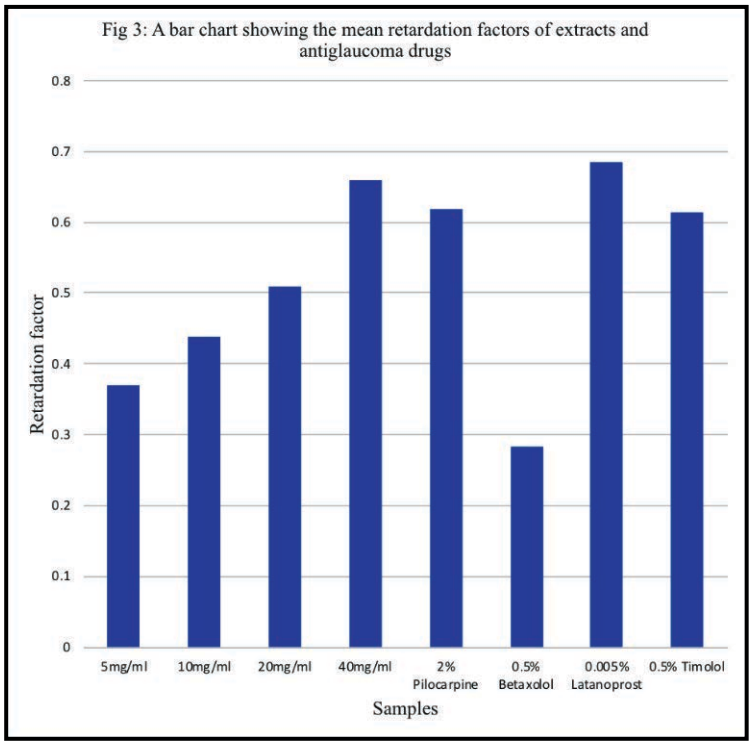


Fig 2: A bar chart representing the average migration distances of the graded concentrations *Pleurotus tuberregium* in comparison with the antiglaucoma drugs.

Table 3: Summary of Plates 1-5, showing the mean retardation factors of various concentrations of PT extract and Antiglaucoma drugs.

Samples	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Mean (R_f) values
5mg/ml	0.366	0.380	0.366	0.373	--------	0.371
10mg/ml	0.413	0.453	0.433	0.440	0.453	0.438
20mg/ml	0.466	0.50	0.533	0.526	0.520	0.509
40mg/ml	0.700	0.653	0.633	0.626	0.670	0.656
2% Pilocarpine	0.613	0.613	0.626	0.626	------	0.619
0.5% Betaxolol	----------		0.266	0.300	-----	0.283
0.005% Latanoprost	----------		0.693	0.680	0.687	0.686
0.5% Timolol	---------		0.620	0.613	0.606	0.613



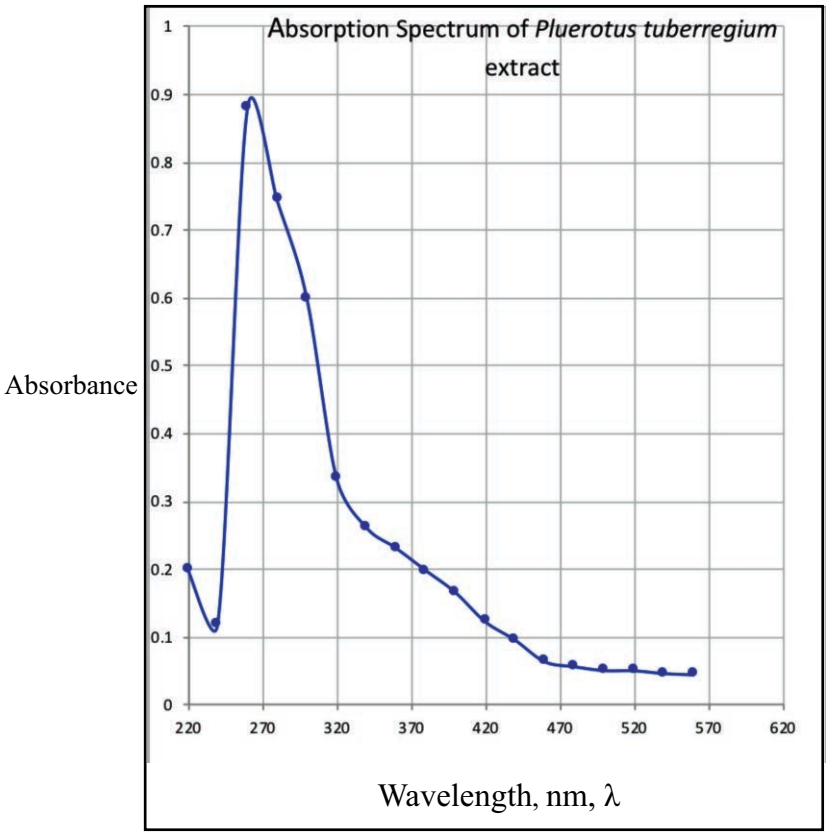


Fig. 4: A plot of the absorption spectrum of *Pleurotustuberregium*

Figure 1 is a bar chart showing the retardation factor of the different concentration of the extract compared to that of known glaucoma drugs as calculated in a sample of the plates (plate 3). The R_f for 40mg/ml of PT extract (0.656) is very close to that of pilocarpine (0.619), timolol (0.613) and latanoprost (0.686). The mean migration distance and retardation factor of the five plates are shown in tables 2 & 3 and their corresponding graphs in Fig. 2 &3.
Spectrophotometry analysis of PT was shown in figure 4, with the plot of absorbance against wavelength. It shows that the maximum absorbance of 0.880 was at 260nm.

DISCUSSION

Pleurotus tuberregium has diverse effects on different organs of the body. Experimental studies on Feline eye models and isolated bovine Iris have proven some of its acclaimed properties and activities. Chromatographic studies on this Mushroom species in comparison with other common and effective antiglaucoma drugs is just timely. There is dearth of literature on spectrophotometric studies on aqueous extracts of *Pleurotus tuberregium*.

This study compared the separation spots of and retardation factors of graded concentrations of *Pleurotus tuberregium* extracts with drugs, whose mode of action or pharmacodynamics are already known and ascertained. The experimental study went a step further by making a plot of the absorption spectrum of *Pleurotus tuberregium* as determined through spectrophotometric studies and compared the wavelength of maximum absorption with the antiglaucoma drugs whose λmax are already known from literature.

Analysis of a compound or extract takes into consideration the qualitative (i.e. identity of the material) and the quantitative (amount of a given substance) present in the extract. TLC takes care of the qualitative aspect while UV spectroscopy gives a semiquantitative measurement.

The results of this chromatographic study of graded concentrations of *Pleurotus tuberregium* in comparison with *2%* Pilocarpine, 0.005 % Latanoprost, 0.5% Timolol and 0.5% Betaxolol has thrown some light as to the pharmacodynamics of *Pleurotus tuberregium.* It is important to mention here that, the pharmacodynamics of a drug is directly related to the active ingredients found in that drug. Hence, the essence of this comparative chromatographic study and spectrophotometric analysis of this Mushroom extract in comparison with known antiglaucoma drugs is to determine the possible existence of similar active ingredients found in the drugs under study, in the Mushroom extract.

In this study, it was found that the retardation factor of Latanoprost is 0.686. This agrees with the findings of a monograph by the United States Pharmacopeia (2012)[18], which stated that the retardation factor of Latanoprost is about 0.7. Also, the migration distance and retardation factor (R_f), of the extract (40mg/ml) on TLC closely resembles that of 2% Pilocarpine, 0.656 and 0.619 respectively; and that of 0.05% Latanoprost, 0.656 and 0.686 respectively. These findings, suggest that there is a possible existence of similarities in the active ingredients of the antiglaucoma drugs in the extracts. This agrees with the study on Bovine Iris sphincter muscle where it caused a contractile effect on the muscarinic receptors of the sphincter pupillae[11].

As stated before, the absorption spectrum of the antiglaucoma drugs under comparison falls within the Ultraviolet range. The absorption spectrum of *Pleurotus tuberregium* also falls within this range with a λmax of about 260nm. Timolol Maleate reduce IOP by decreasing aqueous secretion through blockage of the beta receptors on the ciliary epithelium has an absorption spectrum that falls within the range of the absorption spectrum of *Pleurotus tuberregium* with a λmax of 295nm[19]. Though not a drug under comparison in this study, Brimonidine Tartrate (Alphagan), a relatively selective alpha-2 adrenergic receptor agonist whose

mechanism of action is to reduce aqueous humour production and increase uveoscleral outflow has a λmax of 247.0nm (Popaniya *et a*l., 2014)[20].

Betaxolol chloride, which is a selective beta blocker, decreases aqueous secretion through blockage of the beta receptors on the ciliary epithelium, has an absorption spectrum that falls within the UV range with λmax of 224.0nm[21].

Latanoprost, a prostaglandin analogue, acts on by increasing uveoscleral outflow of aqueous humour thereby reducing intraocular pressure. It is a drug of choice in the management of long- standing cases of primary open angle glaucoma. In a monograph by the United States Pharmacopeia, it has an absorption spectrum within the range of 200-400nm, with a λmax of about 200nm Pilocarpine, which is a parasympathomimetic, acts on the iris sphincter muscles, bringing about pupillary constriction. This opens up the trabecular me, facilitating aqueous humour outflow, it has an absorption spectrum that also falls within the range of *Pleurotus tuberregium,* with a λmax of 215-220nm[22].

It is clear that the absorption spectrum of *Pleurotus tuberregium* falls within the range of the absorption spectra of the antiglaucoma drugs under comparison. It is therefore very likely that the Mushroom extract has similar quantity of active ingredients as the drugs under study and hence similar pharmacodynamic activity.

CONCLUSION

The similarities in the R_f values and λmax (TLC-UVS), qualitative and quantitative analysis) of the extract and the known anti glaucoma drugs has been used as a first-tier structure-activity relationship of the plant extract. This is a preliminary evaluation of the crude aqueous extract of PT, further studies will include the identification, isolation and characterization of the bioactive chemical constituents of PT through activity guided isolation technique.

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